

Effect of Trichloroacetic Acid on Determination of Silicate and Phosphate with Molybdate Reagent

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► Trichloroacetic acid enhances the color obtained with silicate and the molybdate reagent for phosphate (high concentration of sulfuric acid) about fourfold. Because of this, small amounts of silicate can cause serious error in the determination of inorganic phosphate when trichloroacetic acid is present. Trichloroacetic acid also enhances the color obtained with the molybdate reagent for silicate (low concentration of sulfuric acid), but to a considerably smaller degree (26%). In addition, when trichloroacetic acid is present the silicate color is independent of the sulfuric acid concentration and is not influenced by the volume to which the molybdate is added.

THE effect of trichloroacetic acid (TCA) on the molybdate reagent for phosphate (1) or silicate (2) was observed in determining the inorganic phosphate in casein solutions before and after heating. The casein was precipitated with 12.5% trichloroacetic acid and inorganic phosphate was determined in the filtrate without digestion. The apparent quantity of inorganic phosphate in the unheated casein filtrate was much too high, and as found to be due to the presence of a small amount of silicate, which alone was not very reactive with the molybdate reagent for phosphate. The trichloroacetic acid, however, produced a

strong increase (about fourfold) in the color of reduced silicate-molybdate complex. The quantitative results leading to this conclusion are reported because of the importance of the trichloroacetic acid effect for the determination of both phosphate and silicate with the molybdate reagent. This effect may be of analytical usefulness in silicate determination.

REAGENTS

All chemicals used were reagent grade unless stated otherwise.

Sodium caseinate solution, 4%. Prepare by dissolving purified isoelectric casein in water with 0.1N sodium hydroxide (5.5 ml. per 1 gram of casein). The solution had an approximate pH of 6.8.

Trichloroacetic acid (TCA) solution, 25%. Dissolve 100 grams of TCA (sulfate-free) in 400 ml. of water.

The reagents used for the phosphate determination (1, 4) and silicate determination (2) with ammonium molybdate are described in the respective references.

Sodium silicate. Prepare the silicate solution from sodium metasilicate ($\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$) which has been dried at 130° C. to a constant weight. Prepare a stock solution containing 2.4 mg. of the anhydrous sodium metasilicate per ml. and dilute 1 to 240 with water, 24 hours or more before use, to give a standard solution containing 10 γ of

silicate, equivalent to 2.3 γ of silicon per ml.

APPARATUS

The Fisher Electrophotometer, Model AC, was used for colorimetric analysis with the red filter (No. 660) and with matched 23-ml. absorption cells (inside diameter approximately 2 cm.). The Beckman Model B spectrophotometer was also used for colorimetric analysis at a wave length of 650 $\text{m}\mu$ with 18-mm. outside diameter matched absorption tubes.

PROCEDURE

Analytical Methods. Phosphate (and silicate) was measured by the Fiske and Subbarow method (1) with ferrous sulfate as the reducing agent (4). The amounts of each reagent used for digested samples differed slightly from the described procedure and are as follows: 2.5 ml. of 2.5% ammonium molybdate, 1.25 ml. of 7.5N sulfuric acid, and 2.5 ml. of 10% ferrous sulfate, in a final volume of 25 ml. For non-digested samples, 2.5 ml. of 7.5N sulfuric acid should be added. In some experiments, 1 ml. of aminonaphtholsulfonic acid (ANSA) was used as the reducing agent (2), replacing the ferrous sulfate.

Silicate was determined by the method described by Kenyon and Bewick (2). The amounts of reagents used in the present study were the same as in the phosphate method above, except that only 0.1 ml. of sulfuric acid

was used instead of 2.5 ml. The amount of acid used is the essential difference between the two methods. Silicate gives very little color in the phosphate method, but both phosphate and silicate give the same amount of color with the silicate method.

Precipitation of Casein. The sodium caseinate solutions were precipitated with acid by adding an equal volume of 25% TCA to the 4% casein solution, or by adding 0.1N hydrochloric acid equal to the amount of 0.1N sodium hydroxide present in the casein solution and bringing the concentration of casein to 2% with water. The solutions were centrifuged at 3500 r.p.m. for 5 minutes, then filtered through a pad of glass wool to remove floating particles of casein. Analytical determinations were performed with this filtrate.

Digestion. Samples (0.5 ml.) were digested by heating with 1 ml. of 18.3N sulfuric acid until dense sulfur trioxide fumes appeared. A few drops of 30% hydrogen peroxide were added to complete the digestion.

RESULTS

A source of error in the inorganic phosphate determination in acid filtrates of casein was apparent when the values obtained were much too high. For example, when TCA was used as the precipitant, filtrate corresponding to 1 ml. of a 2% casein solution contained 36.4 γ of apparent phosphorus (Table I), equivalent to 22.7% of the phosphorus in the casein. When hydrochloric acid was the precipitant, the phosphorus value was less, but still considerable (Table I). When the filtrates were digested with sulfuric acid, the amounts of phosphorus were now negligible and of the order of 1 to 2 γ . This was further evidence that the color values obtained with the undigested samples did not represent phosphate. Further, the enhancing effect of TCA was not apparent with a phosphate standard (Table I). Determination of the nitrogen content in the filtrates showed that very little of the original casein is in either the TCA or the hydrochloric acid filtrates. As the amount of nitrogen is about the same in each, the large amount of color in the TCA filtrates could not be attributed to casein ester phosphate. Further, when TCA is added to the hydrochloric acid filtrates, additional color is obtained. Digesting the sample reduced the color to a low level. The results were the same whether the TCA was added before or after the sample was digested.

When the amount of hydrochloric acid filtrate was varied (Table I) and TCA addition kept constant, the color values were linear in respect to amount of filtrate. In determining the nature of the substance in the filtrate giving the enhanced molybdate color with

Table I. Effect of Trichloroacetic Acid and Digestion of Sample^a
(Apparent inorganic phosphate measured with molybdate reagent in acid filtrates of 2% casein solutions)

Sample	Casein Equiv., Ml. of 2% Soln.	Apparent Inorganic Phosphate ^b				Nitrogen ^c
		Sample as is	Digested	TCA added	TCA added to digested	
TCA filtrate	1.0	36.4	2.3	16
	1.0	38.5	1.1	10
HCl filtrate	1.0	10.0	2.0	45.3	2.0	10.5
	1.0	8.1	1.1	44.8	1.1	10.5
	0.5	21.4
	1.0	37.4
	2.0	78.0
HCl filtrate, fresh NaOH ^d	1.0	3.8	...	4.7
Phosphate, 25 γ		25.0	25.0	24.6

^a Data obtained with Fisher Electrophotometer; a reading of 11.1 is taken equal to 25 γ of phosphorus. Final volume in these experiments was 50 ml.

^b Expressed as γ of phosphorus per sample.

^c Expressed as γ per sample. As 1.0 ml. of a 2% casein solution contains 3.0 mg. of nitrogen, a filtrate containing 16 γ of nitrogen contains only 0.5% of the total nitrogen.

^d NaOH used to prepare the casein solution.

Table II. Effect of Trichloroacetic Acid and Concentration of Sulfuric Acid on Color Obtained with Silicate and Molybdate Reagent
(ANSA used as reducing agent)

Na ₂ SiO ₃ , Si Equiv., γ	H ₂ SO ₄ , Ml. ^a	Spectrophotometer Reading ^b			
		No TCA		TCA Added ^c	
		Per 10 γ		Per 10 γ	
		In 0.75N H ₂ SO ₄ (Phosphate Method)			
6.9	2.5	0.032	0.046	0.116	0.168
11.5	2.5	0.059	0.051	0.202	0.176
16.1	2.5	0.075	0.047	0.266	0.166
23.0	2.5	0.110	0.048	0.401	0.174
27.6	2.5	0.130	0.047	0.480	0.174
		Av. 0.048			0.172
		In 0.030N H ₂ SO ₄ (Silicate Method)			
6.9	0.1	0.091	0.132	0.116	0.168
11.5	0.1	0.153	0.133	0.192	0.167
16.1	0.1	0.211	0.131	0.271	0.168
23.0	0.1	0.309	0.134	0.383	0.167
27.6	0.1	0.450	0.163
		Av. 0.133			0.167
		In Variable H ₂ SO ₄			
23.0	0	0.076	0.033	0.365	0.159
	0.05	0.247	0.107	0.389	0.169
	0.1	0.317	0.138	0.392	0.170
	0.5	0.365	0.159	0.430	0.187
	1.0	0.185	0.080	0.392	0.170
	2.0	0.135	0.059	0.391	0.170
	3.0	0.130	0.056	0.392	0.170

^a Ml. of 7.5N H₂SO₄ in volume of 25 ml.

^b In Beckman spectrophotometer, final volume 25 ml.

^c 0.25 ml. of 25% solution.

TCA, a number of factors were investigated. Even though the quantity of casein that might be present was small (nitrogen content 10 to 16 γ), this was considered as a possibility. Addition of small amounts of sodium caseinate (20 γ per ml.) to standard phosphate solutions, both with and without TCA present, gave the same color values as the phosphate standard alone with the molybdate reagent. Also tested were mixtures of the amino acids occurring in proteins, and again the results were negative. A phosphopeptide from casein showed no color with or without TCA present. In attempting to find the cause of the

TCA effect, a fresh solution of 0.1N sodium hydroxide was prepared. With the fresh solution, the unknown factor was not obtained, suggesting sodium silicate contamination of the sodium hydroxide solution first used. Consequently, a solution of sodium silicate was prepared and tested for the TCA effect with the phosphate method (Table II). Addition to TCA caused a 3.6-fold increase in color obtained with the molybdate reagent. Digestion of the sodium silicate solutions with sulfuric acid all but completely eliminated the color and TCA effect. Using the silicon color equivalent, it was estimated that the original

sodium hydroxide used in preparing the casein solution contained about 0.5 mg. of sodium silicate per ml. A direct determination, after neutralization with hydrochloric acid, of the silicate concentration in the sodium hydroxide used confirmed this value. This solution had been stored in a glass bottle for about 6 months.

As the striking enhancement of the color obtained with the molybdate reagent and silicate in the presence of TCA suggested that this procedure might be analytically useful, silicate was determined with both the system used for phosphate (1) and the method devised for silicate (2) with TCA present. Also, the concentration of the sulfuric acid was varied to determine the effect of intermediate concentrations. Results are shown in Table II.

With the silicate method, the increase in color with TCA present is only 26%; however, both phosphate and silicate methods gave about the same amount of color with TCA present. Varying the sulfuric acid showed the striking dependence of the silicate color on the acid concentration. When TCA is present, however, the color is no longer dependent on the sulfuric acid concentration. TCA did not increase the color with phosphate with either a high or low concentration of sulfuric acid.

The effect of the volume containing the sample to which the molybdate reagent is added on the final color, reported by Kenyon and Bewick (2), was confirmed. The smaller the volume containing the silicate sample when the molybdate is added, the greater the final color, but it is also less reproducible. The effect of volume is

not observed, however, when TCA is present.

The concentration of TCA regularly added in the analyses was 0.25 ml. of a 25% solution. Smaller amounts gave proportionately less color. Doubling this concentration gave only a 4% increase in the color of the final mixture. Acetic acid also increased the color obtained with silica and the molybdate reagent; however, at equivalent concentrations it had only about one half the effect of TCA.

DISCUSSION

The leaching of silicate from glass bottles by alkaline solutions is well known, but the present studies emphasize the large amount. In examining three solutions of 0.1N sodium hydroxide stored in glass bottles, the sodium silicate found was 0.3, 0.5, and 0.7 mg. per ml. The molybdate reagent for phosphate is used at high acidity to give a minimum reaction with silicate (1); however, some color will be obtained with silicate and, as observed in the present study, can be a source of large error when inorganic phosphate is determined. When total phosphate is determined and the samples are digested, the silicate will not interfere, as it is converted to an inert substance, silicon dioxide or colloidal silica. The interference of silicate in the phosphate determination is especially serious when trichloroacetic acid is present, because this reagent increases the silicate color with the molybdate reagent fourfold.

The effect of TCA on the color given by silicate with the molybdate reagent may be useful analytically. The color enhancement is obtained not only with

the strongly acid molybdate reagent used for phosphorus (260% increase), but also with the less acid reagent used for silicate (26% increase). Further, when TCA is present, the acidity need not be controlled as precisely, nor is the volume (concentration of reactants), to which the molybdate solution is added, as important. The TCA effect on the molybdate reaction with phosphates or silicates should also serve to distinguish these compounds qualitatively in unknown mixtures.

The specificity of the TCA for the silicate-molybdate reaction is very striking, as the effect is not obtained with phosphate. The nature of the TCA effect can only be surmised at present, although it is observed in the formation of the yellow silicate-molybdate complex and persists in the reduction to the blue molybdate complex. Strickland (3) has shown that several forms of the silicate-molybdate complex can be prepared. Measurements of the light absorption of the complex with and without TCA at 400 to 800 m μ suggest that TCA might further the α - to β -complex transformation described by Strickland (3). A shift in the silicate-molybdate equilibrium might also be involved.

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